AD

TECHNICAL MANUSCRIPT 279

THE EFFECT OF ETHYLENE ON AUXIN TRANSPORT

Frederick B. Abeles

JANUARY 1966

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 279

THE EFFECT OF ETHYLENE ON AUXIN TRANSPORT

Frederick B. Abeles

Crops Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1L014501A91A

January 1966

ACKNOWLEDGMENTS

I wish to acknowledge the aid of Sp4 A. Hartman, Lt. R. Holm, and Lt. T. Weymouth during the course of these experiments.

ABSTRACT

Ethylene was found to have no effect on auxin transport in hypocotyls, coleoptiles, or leaf petioles.

I. INTRODUCTION

Zimmerman and Wilcoxon[1] were the first to show that auxin stimulated ethylene synthesis. Since that time we have reported[2,3] that endogenous as well as exogenous levels of auxin may affect ethylene biosynthesis. However, the reverse effect, that of ethylene on auxin metabolism and physiology, has been reported in relatively few papers.[4-6] The equivocal results of some of these earlier papers are discussed later.

We are reinvestigating the effect of ethylene on auxin transport because others have used an inhibition of transport to explain the physiological effects of ethylene. For example, Hall and Morgan[7] found that greater amounts of indoleacetic acid (IAA) oxidase were extracted from ethylene-treated vs. control plants. They postulated that the increased activity of the oxidase prevented the auxin from reaching the abscission zone and that lowered auxin levels in turn permitted leaf abscission. By measuring auxin transport through a variety of plant structures such as coleoptiles, hypocotyls, and petioles, it should be possible to determine if ethylene enhances oxidase levels in vivo. If increased levels of oxidase are acting in ethylene-treated sections, then less auxin should be collected from the bottom of treated sections. Inhibition of growth[4] and geotropism[8] by ethylene are other examples that might implicate an effect of ethylene on auxin transport.

In this report we will show that ethylene has no significant effect on auxin transport.

II. MATERIALS AND METHODS

Five plant species were chosen for the experiment—Zea mays L. var. Burpee's Snowcrop (corn), Helianthus annuus L. var. Mammoth Russian (sunflower), Gossypium hirsutum L. var. Acala 4-42 (cotton), Phaseolus vulgaris L. var. Red Kidney (bean), and Coleus blumei Benth. (coleus). The methods of growing and collecting corn and sunflower were almost identical with those described by Gillespie and Thimann.[9] Cotton and bean plants were grown in soil in 10-cm pots under 1200 foot candles of a combination of fluorescent and incandescent lights for 14 hours per day at 27 C. Coleus plants were grown in the greenhouse in soil. Etiolated beans were grown on moist vermiculite at 25 C and harvested after 7 days.

Petiole sections were collected from the middle of the petioles of (i) the primary leaf of 2-week-old beans; (ii) the cotyledonary leaf of 2-week-old cotton plants; and (iii) the fourth leaf of coleus leaf number one being the uppermost with a petiole of 5 mm or more. Hypocotyl tissue from etiolated beans was collected just below the crook.

The beta-indoleacetic-2 C^{14} acid (C^{14}IAA) (specific activity 0.96 microcurie per mole) was used as obtained from Baird Atomic Inc., Cambridge, Massachusetts. The purity of the compounds was checked by paper chromatography[10] and the radioactivity of the spots was assayed with a strip scanner. Ninety-five per cent of the activity was associated with a Salkowski-positive spot that had a R_f similar to that of pure IAA.

Agar disks (1.5%) containing C^{14}IAA were prepared according to the method of McCready.[11] Activity of the agar blocks and tissue was measured by a windowless flow counter. The method of preparing samples for counting followed essentially that of McCready.[12]

Sections of tissue 5.7 mm long, apical or distal end upward, were set on plain agar disks (height 1.5 mm, diameter 5 mm). Another agar disk of similar dimensions that contained 6×10^{-10} mole of C^{14}IAA was set on top of the tissue sections. This concentration of C^{14}IAA was the lowest that would give significant counts in the receiver blocks. Higher concentrations were not used because they might interfere with endogenous transport or give rise to a stimulation of endogenous ethylene evolution. Others[9] have shown that the concentration used here is within the physiological range. During the time allotted for these experiments no ethylene was observed in the gas phase above the control sections. The gas chromatograph technique used to measure ethylene is capable of measuring levels as low at 0.05 ppm.

Ten sections with donor and receiver blocks were set in a glass container that was sealed with a rubber vaccine cap. When required, 1 ppm ethylene was injected into the bottles after the last of the sections was set up. This concentration is maximal for most effects of ethylene on physiological processes such as growth, abscission, and fruit ripening.[2,4] Higher concentrations usually do not have any additional effect. Moist filter paper was placed inside the bottles to prevent the agar blocks and tissue from drying out. After 3 hours, donor blocks, receiver blocks, and tissue sections were collected and pooled into three separate planchets and counted. Evidence that the radioactivity is associated with the IAA in the receiver blocks has been discussed by Goldsmith and Wilkins.[13] Each experiment with three controls and three ethylene-treated containers was repeated three times except those with coleus, which were repeated twice. Data are presented as the mean of each group of three, plus or minus the standard deviation.

III. RESULTS

Table 1 summarizes a series of experiments designed to measure the effect of ethylene on polar auxin transport. There was no observable difference between treated and control sections using coleoptiles, hypocotyls, or petioles from a variety of plants. In addition, ethylene had no effect on the amount of activity left in the donor block or incorporated in the tissue sections. Neither greater concentrations of ethylene (5 ppm) nor measurements of transport up to 24 hours produced data different from those presented here. Some experiments were performed measuring the effect of ethylene on nonpolar transport (donor block basal) on corn coleoptiles and bean hypocotyls. Little transport was observed (about 1 to 2%) under these conditions, and ethylene had no effect on the amount of activity observed.

TABLE 1. EFFECT OF ETHYLENE ON AUXIN TRANSPORT
B-INDOLEACETIC-2-C^{14} ACID[a]

Plant Material	% Total Initial Radioactivity in Receiver Blocks[b]	
	Control	Ethylene
Zea mays coleoptiles	26.6±4.4	31.2±7.9
	23.8±0.2	26.0±5.8
	18.6±4.1	18.3±2.6
Helianthus annuus hypocotyl	7.8±1.2	3.8±1.4
	3.8±0.2	3.4±0.3
	4.8±1.5	5.8±3.0
Phaseolus vulgaris hypocotyls	15.9±7.2	19.6±8.4
	16.8±5.9	12.6±4.5
	16.7±6.0	15.6±0.6
Gossypium hirsutum petioles	3.5±2.5	4.1±1.2
	2.9±0.9	3.4±1.0
	2.5±1.0	3.0±1.4
Phaseolus vulgaris petioles	2.6±0.2	2.6±0.8
	3.0±1.0	1.8±0.1
	2.0±1.2	1.3±0.6
Coleus blumei petioles	2.8±1.0	2.8±1.6
	1.3±1.1	1.1±0.2

a. Experimental conditions: Specific activity of C^{14}IAA; 0.96 μc/mmole, 6 x 10^{-10} mole in 60 mm^3 1.5% agar disk. Ethylene; 1 nl/ml gas phase, total gas phase 41 ml. Duration of experiment, 3 hours. Temperature, 25 C.

b. Plus or minus standard deviation.

IV. DISCUSSION

The first worker to report on the effect of ethylene on auxin transport was Van der Laan.[e] He found that between 5 and 50 ppm ethylene had no effect on auxin-a (not IAA) transport through Avena coleoptiles. Later Michener[6] obtained conflicting results, depending on how the experiment was performed. He reported that IAA transport through coleoptile sections harvested from ethylene-treated and control plants was the same. However, in another experiment, pea seedlings were decapitated and a lanolin paste containing IAA was applied to the top. After three hours' transport in the presence of 1,000 ppm ethylene, they were assayed for auxin content; he found that less auxin was present in the subtending tissue of the plants exposed to ethylene. More recently, Burg and Burg[4] have shown in experiments similar to those reported here that ethylene had no effect on C^{14} auxin transport through pea stem sections.

During the course of our own experiments we failed to detect any effect of ethylene on auxin transport through hypocotyls, coleoptiles, and petioles of the five plant species tested. On the basis of our results, we believe that the action of ethylene on plant processes such as leaf abscission, inhibition of growth, and inhibition of geotropism will have some explanation other than an interference with polar auxin transport.

LITERATURE CITED

1. Zimmerman, P.W.; Wilcoxon, F. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson In... 7:209-229.

2. Abeles, F.B.; Rubinstein, B. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39:963-969.

3. Abeles, F.B. July 1965. Auxin stimulation of ethylene evolution, (Technical Manuscript 234). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. Also, Plant Physiol. (In press).

4. Burg, S.P.; Burg, E.A. 1965. Ethylene action and the ripening of fruits. Science 148:1190-1196.

5. Michener, H.D. 1938. The action of ethylene on plant growth. Amer. J. Bot. 25:711-720.

6. Van der Laan, P.A. 1934. Der Einfluss von Aethylen auf die Wuchsstoffbildung bei Avena und Vicia. Rec. Trav. Bot. Neerl. 31:691-742.

7. Hall, W.C.; Morgan, P.W. 1964. Auxin ethylene interrelationships, p. 727-745. In Regulateurs naturels de la Croissance vegetale. Gif s/Yvette. 1963. 15, Quai Anatole-France, Paris (VII^{e}).

8. Doubt, S.L. 1917. The response of plants to illuminating gas. Botan. Gaz. 63:209-224.

9. Gillespie, B.; Thimann, K.V. 1963. Transport and distribution of auxin during tropistic response: I. The lateral migration of auxin in geotropism. Plant Physiol. 38:214-225.

10. Stowe, B.B.; Thimann, K.V. 1954. The paper chromatography of indole containing auxins of plant tissues. Arch. Biochem. Biophys. 51:499-516.

11. McCready, C.C. 1963. Movement of growth regulators in plants: I. Polar transport of 2,4-dichlorophenoxyacetic acid in segments from petioles of Phaseolus vulgaris. New Phytol. 62:3.

12. McCready, C.C. 1958. A direct plating method for the precise assay of C^{14} in small liquid samples. Nature 181:1406.

13. Goldsmith, M.H.M.; Wilkins, M.B. 1964. Movement of auxin in coleoptiles of Zea mays L. during geotropic stimulation. Plant Physiol. 39:151-162.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY *(Corporate author)*

U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland, 21701

2a REPORT SECURITY CLASSIFICATION

Unclassified

2b GROUP

3 REPORT TITLE

THE EFFECT OF ETHYLENE ON AUXIN TRANSPORT

4 DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5 AUTHOR(S) *(Last name, first name, initial)*

Abeles, Frederick B.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
January 1966	12	13

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO. 1L014501A91A	Technical Manuscript 279
c.	9b OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10 AVAILABILITY/LIMITATION NOTICES

Qualified requestors may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701

13 ABSTRACT

Ethylene was found to have no effect on auxin transport in hypocotyls, coleoptiles, or leaf petioles.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification